IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Sydney M. Pugh et al.		
Title:	ARTIFICIAL STABILIZED COMPOSITION OF CALCIUM PHOSPHATE PHASES PARTICULARLY ADAPTED FOR SUPPORTING BONE CELL ACTIVITY		
App. No.:	09/029,872	Filed:	June 29, 1998
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MS AMENDMENT
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

1. I am a joint inventor of the subject matter presently claimed in the above-identified patent application.

2. I received a Ph.D. of Biomechanics and Applied Science in 1988 at Queen's University of Kingston, Ontario, Canada. In addition, I received a M.Sc. of Biomechanics and Applied Science in 1985 at Queen's University of Kingston, Ontario, Canada.

3. I have over 20 years experience in the medical device field. For much of my career, I have been employed in technical programs relating to bone biomaterials and tissue engineering. Specifically, I was a co-founder of Millenium Biologix, Inc., where I jointly invented the subject matter presently claimed. Subsequently, I became co-founder and CEO of Octane Orthobiologics, Inc., where I continue to work in the bone biomaterials and tissue engineering fields.

4. I have over 20 technical publications in the areas of biomaterials and biomechanics. In addition, I am a joint inventor of at least 15 patents related to bone biomaterials arts.

5. I have reviewed the Ruys reference (Ruys, A. J. Silicone-Doped Hydroxyapatite. J. Aust. Ceram. Soc., 29 (1/2), 71-80 (1993).) and the present specification (US Patent Application no. 09/029,872) in detail. As a result, I noted several differences in the disclosed methods and several differences in the resulting materials.

Ruys Method

Ruys discloses forming a $Ca_3(PO_4)_2$ precipitate from $Ca_3(NO_3)_2$, $(NH_4)_2HPO_4$, and NH_4OH. Note that $Ca_3(NO_3)_2$ does not exist. For the sake of discussion, I will presume that Ruys means $Ca(NO_3)_2 \cdot 4H_20$ instead of $Ca_3(NO_3)_2$.

The precipitate is stir/boiled for 24 to 48 hours to eliminate TCP from the calcined product. (Ruys, pg. 74). This process is generally called "aging," and represents an important step in the formation of the material. Ruys fails to provide key parameters related to aging.

According to Ruys, the resulting hydroxyapatite crystals were washed twice by filtering and resuspending in demineralized water. After the second washing, the wet filter cake was resuspended in ethanol. (Ruys, pg. 74). A solution of tetraethyl orthosilicate (TEOS) was added to the hydroxyapatite/ethanol suspension, and the suspension was subjected to high-speed stirring. Excess water was added to hydrolyse the ethyl silicate, followed by additional high-speed stirring. (Ruys, pg. 76).

The ethanol was removed by evaporation. The resulting filter cake was crushed and pelletised. The pellets were sintered at 1100°C for 1 hour in air with a heating rate of 60°C/h. (Ruys, pg. 76). While Ruys specifies the sintering temperature and heating rate, Ruys fails to discuss cooling of the sintered material. In my experience, cooling rate and other cooling conditions can influence the crystal composition and morphology of a sintered material.

While attempts were made to follow the processes in the Ruys reference, I was unable to reproduce the material of Ruys over a several month period, even after attempting modifications based on numerous literature references to enable successful material synthesis. Without the noted additional parameters that are not easily ascertained from the Ruys reference and those references to which Ruys refers, it would require an excessive amount of experimentation to reproduce the material of Ruys.

Present Method

The method disclosed in the present application is significantly different from that of the Ruys reference. In particular, the method disclosed in the examples of the present specification includes formation of a hydroxyapatite suspension. (Present Specification, pg. 28, Procedure 1). In contrast to the ethanol suspension of Ruys, the present hydroxyapatite suspension is aqueous. Further, the method disclosed in the present application is less sensitive to aging conditions.

In addition, the present method prepares a silicon solution of tetrapropyl orthosilicate and 2-methoxyethanol. The silicon solution is added to the aqueous hydroxyapatite solution. (Present Specification, pg. 29, Procedure 2). Tetrapropyl orthosilicate generally has better dispersion in aqueous solutions than TEOS and provides for more uniform dispersion of silica resulting from the addition of the silicon solution to the aqueous hydroxyapatite solution. The resulting solution is dried at 100°C (Present Specification, pg. 31, Procedure 4) and sintered at between 920°C and 1100°C. (Present Specification, pg. 32, Procedure 7).

As noted in the present specification, selection of a sintering temperature between 920°C and 1100°C results in compositions having desirable ratios of α-tricalcium phosphate relative to hydroxyapatite. In particular, the present specification discloses sintering at temperatures less than 1100°C, such as between 950°C and 1000°C. In fact, the present specification discloses that the formation of α-tricalcium phosphate is enhanced at temperatures below 1000°C. In contrast, Ruys discloses sintering at 1100°C.

Compositional Differences

The differences between the method of Ruys and that disclosed in the present specification lead to important differences in the resulting compositions. Such differences manifest in both phase composition and cellular/physiological response to such a composition.

As reported by Ruys, a high ratio of Si to hydroxyapatite is required in order to produce a composition having slightly greater amounts of tricalcium phosphate than hydroxyapatite. However, Ruys reports that at such high Si to hydroxyapatite ratios the dominant phase of the composition is Si-P-O glass. (Ruys, pg. 77).

In my studies of such compositions, such a high concentration of silicon external to a calcium phosphate crystal lattice prevents proper cell attachment to the surface of the composition when the composition is implanted *in vivo*. Generally, calcium phosphate compositions having greater than 20wt% silicon exhibit biological performance that is inferior to compositions of reduced silicon content. In particular, such high silicon content results in reduced osteoblast attachment with a related reduction in new bone formation, plus reduced osteoclast activity due the imposed high metabolic loading of silicon incurred under conditions of cell-mediated remodeling. As such, a high silicon level prevents balanced osteoclast and osteoblast activity according to the claimed invention.

In contrast, compositions including lower amounts of silicon exhibit enhanced bone mineralization followed by de-novo bone formation. As noted by Thian et al. (Thian, Eng San, Huang, Jie, Best, Serena M., Barber, Zoe H., Brooks, Roger A., Rushton, Neil, Bonfield, William . The response of osteoblasts to nanocrystalline silicon-substituted hydroxyapatite thin films. Biomaterials 27 (2006) 2692-2698) and by Best et al. (Best, S. M., Zou, S., Brooks, R., Huang, J., Rushton, N., and Bonfield, W. The osteogenic behaviour of silicon substituted hydroxyapatite. Key Engineering Materials 361-363 (2008) pp. 985-988.), the optimal silicon substitution is less than 5.0 wt% and more typically, around 2.2 wt% or 0.8 wt%.

The material reported by Ruys is predominantly hydroxyapatite at low silicon loading. However, at high silicon loading, the material is predominantly Si-P-O glass. (Ruys, pg. 77). In

addition, there may be slightly greater alpha tricalcium phosphate than hydroxyapatite, the combination forming a lesser phase. From Ruys' description, it is clear that the resulting material has significantly greater than 20wt% silicon and thus, would exhibit unbalanced bone cell activity. In fact, based on the description of "dominant phase", the silicon level is calculated to be at least 20wt% and likely in a range of 25wt% to 45wt%.

In contrast, compositions disclosed in the present specification have lower loadings of silicon, generally less than 5 wt%, to preserve balanced bioactivity. Moreover, we discovered compositions including stabilized alpha tricalcium phosphate at lower silicon loading, which exhibit balanced growth. In particular, such compositions are demonstrated to permit osteoclast activity. (See Present Specification, pg. 25, ll. 12-22; FIG. 9; and FIG. 13).

Conclusion

In summary, the Ruys reference lacks key disclosure of process steps, such as mixing and cooling conditions, making it difficult to reproduce the material of Ruys. Nevertheless, there are significant differences between the processes of Ruys and that disclosed in the present specification that lead to important differences in the materials produced by such methods. In particular, the silicon levels are different in the materials, resulting in different *in vivo* responses.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like, so made, are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

NOVEMBER 2, 2007

Date

Timothy J. N. Smith, Ph.D.